RESPONSES OF DEEP-SEATED MELANOPHORES IN FISHES AND AMPHIBIANS

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Introduction

Although there have been numerous investigations of the activities of the skin melanophores (Parker, 1930), only a few have included the deep-seated melanophores. These (Lieben, 1906; Ogneff, 1908; Hooker, 1912; Allen, 1917; Smith, 1916; Fischel, 1920; Uyeno, 1922; Gilson, 1926; Yamamoto, 1931) suggest little correlation in the activities of the two sets of melanophores. In amphibians, the absence of any marked response in the deep-seated cells to such drugs as adrenalin may have been due to a degenerate condition, since most of the earlier work was carried out after killing the animal. In fish, various reactions have been found, some identical to those in the skin, others the reverse. Because of these results, it seemed well to examine the activities of the deep-seated melanophores in animals in which the behavior of the dermal melanophores had been studied, by the use of the same agents which affect these cells in the skin, to learn whether the reactions of the deep-seated melanophores are comparable with those in the skin. The animals chosen were the common leopard frog, *Rana pipiens* Schreber, the tadpole of *Rana clamitans* Latreille, and the killifish, *Fundulus heteroclitus* L.

This work was carried out at the suggestion of Professor G. H. Parker to whom I am indebted for his constant supervision and advice.

Methods

The three species used, on a black background assume a dark coloration by expansion of their dermal melanophores, and on a white one, a light shade by contraction of these cells. Such conditions may also be induced by hormones, adrenalin producing a light appearance and pituitrin, at least in amphibians, a dark one. In extending these tests to the deep-seated melanophores, white porcelain dishes served for one extreme of background and battery jars painted externally with dull black, the other. In adrenalin tests, the animals were confined in the black jars, and in the pituitrin tests, they were placed in the white dishes, some time before injecting and kept there until they were killed. Throughout

all experiments the animals were illuminated from a western window or by an electric lamp.

As the condition of the deep-seated melanophores may be inspected only upon killing the individual, a group of animals was subjected to the various tests and the internal melanophores of a number of these were examined at intervals during such trials to trace the activities of these cells step by step. Caution was taken that no rearrangement in the

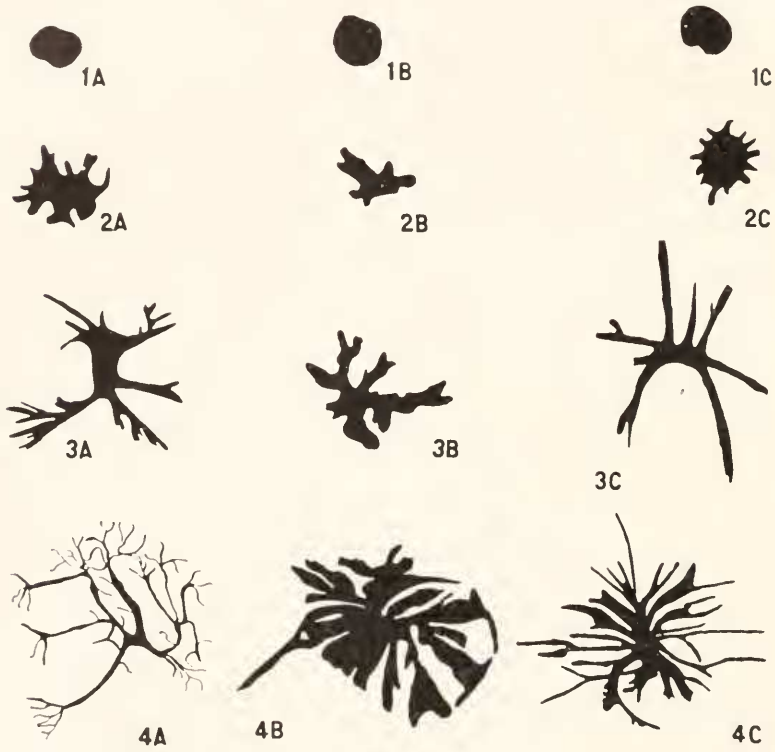

PLATE I

Semidiagrammatic drawings of dermal melanophores from the frog (*A*), the tadpole (*B*), and the killifish (*C*) showing four stages of expansion: 1, punctate condition; 2, stellate condition; 3, incomplete expansion; 4, complete expansion.

position of the pigment within the internal cells occurred during dissection. In frogs and tadpoles, whose dermal melanophores are chiefly under humoral control, no perceptible change appeared after considerable handling or for some hours after pithing. Hence, the state of the internal melanophores within a minute or two of pithing seemed indicative of the living condition. However, in killifish, whose melanophores are almost entirely under nervous control, these cells expanded at once

on manipulation and especially on sectioning their nervous connections. To overcome this, the deep-seated tissues were examined without first destroying the brain, by cutting away the body wall on one side without

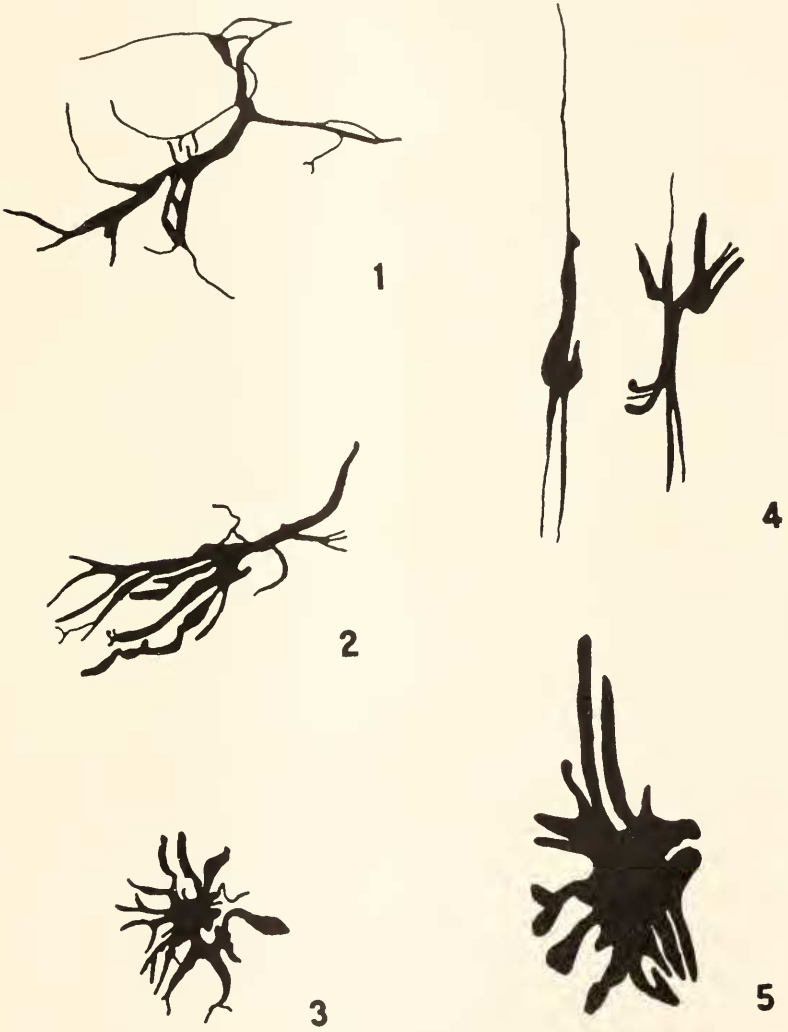

PLATE II

Semidiagrammatic drawings of completely expanded melanophores of the frog in (1) the pleura, (2) the mesenteries, (3) the fascia of the leg muscles, (4) the fascia of the body-wall muscles, and (5) the fascia of the back muscles.

severing the nerves to the parietal peritoneum of the opposite wall or to the visceral peritoneum and mesenteries.

To facilitate a comparison of conditions among the deep-seated me-

lanophores, four conventional stages have been defined as follows: (1) the *punctate* stage, an entirely contracted state in which the cell appears as a minute black dot; (2) the *stellate* stage, or one in which the pigment occupies only the main roots of the cell processes; (3) the stage of *incomplete expansion*, in which the melanin partly fills these; and (4) the stage of *complete expansion*, in which the pigment has spread into the most remote branches (Pl. I).

TABLE 1

The variation in the degree of contraction among the melanophores of frogs confined in darkness or on a white illuminated background.

Location of melanophores	Case a74— Kept in dark 20 hours	Case a107— White illuminated surroundings 24 hours	Case a190— White illuminated surroundings 2 weeks
Skin on back	Stellate	Stellate	Stellate
Web	Stellate	Stellate	Stellate
Back muscles	Punctate to stellate	Punctate to incomplete expansion. Mostly stellate.	Punctate
Pleura	Stellate	Punctate	Punctate to incomplete expansion
Pericardium	Punctate to incomplete expansion	Stellate to incomplete expansion	Incomplete expansion
Muscles of body wall	Punctate to stellate	Punctate	Punctate to stellate
Mesenteries	Chiefly punctate to incomplete expansion	Punctate	Punctate

OBSERVATIONS

Frogs

In frogs, the deep-seated melanophores are particularly abundant in the connective tissues of the following regions: the fascia of the leg muscles, of the muscles of the back, of the muscles of the body wall—especially the m. obliquus internus—, the mesenteries, the pericardium, the pleura, and the lining of the sub-dermal lymph sacs. These cells are very similar in general appearance to the melanophores of the skin, with the exception of those in the fascia of the back muscles, which resemble those in tadpole connective tissues (Pl. II).

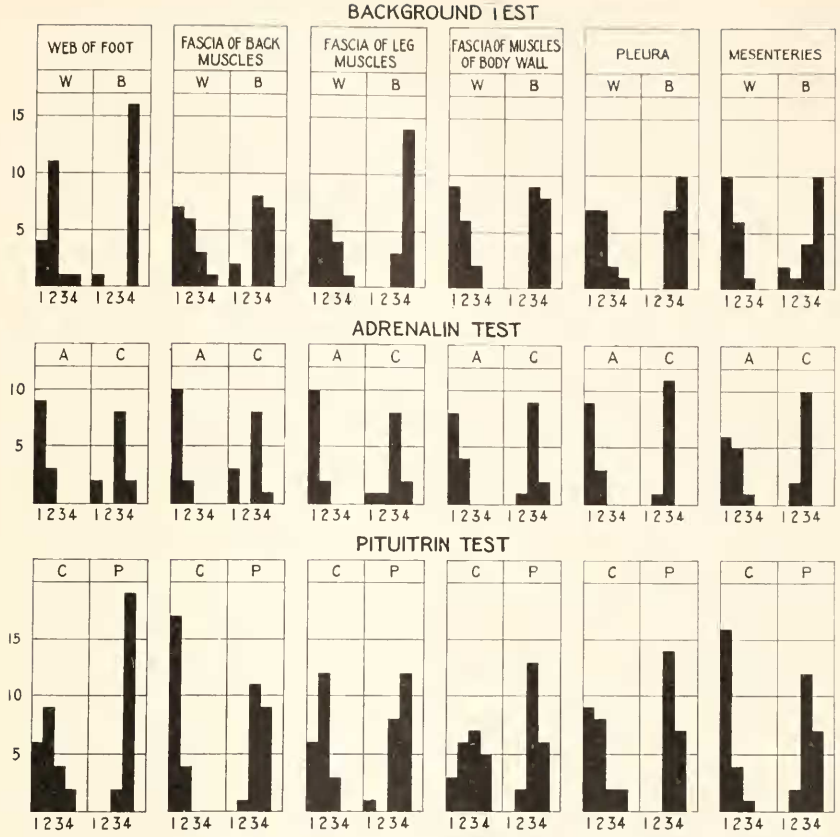

Diagram 1. Tabulation of the effects of background, adrenalin, and pituitrin on the melanophores in six different parts of the frog: the web of the foot, the fascia of the back-muscles, of the leg muscles, and of the muscles of the body-wall, the pleura, and the mesenteries (as shown at the top of the diagram). In the background tests, the shade of the surroundings, white or black, is indicated by *W* or *B* at the head of each column in the upper row. In these tests, as in all the subsequent ones, the results of the factor effecting the more complete contraction are placed to the left and of that producing the less complete to the right in each contrasting pair.

Seventeen frogs were kept on each background for forty-eight hours before observation. In every frog, the amount of melanophore contraction was noted as belonging to one of four stages of contraction of which 1 is maximum contraction and 4 is minimum contraction. The number of cases falling in each class is recorded in black columns over the appropriate figure. Thus, in the web of the foot of frogs on a white background, four animals showed complete contraction, eleven part contraction, one part expansion, and one full expansion: on a black background, one exhibited full contraction and sixteen complete expansion. The remaining tests are all tabulated in a similar manner.

In the adrenalin test, the frogs treated with a 0.5 cc. injection of the drug three hours before examining and their controls with 0.5 cc. tap water injections are designated *A* or *C* respectively at the top of each column in the second row. The twelve frogs in each series were kept on an illuminatd black background for two days before observation.

In the pituitrin tests, the frogs subjected to a 0.5 cc. pituitrin injection three hours before examination and their controls with 0.5 cc. of tap water are distinguished in the diagram by *P* or *C* at the top of each column in the bottom row. The twenty-one frogs in each series were confined in complete darkness for several days before their inspection.

The deep-seated melanophores of frogs confined on a black background for periods extending from a day to a week were predominantly expanded. Yet there were always some cells among them in stages of contraction. This situation was unlike that in the skin where the melanophores were always in a uniform state of expansion.

TABLE II

The rate of response in melanophores to adrenalin. Frogs kept in blackened battery jars under illumination before the injection and until killed.

Location of melanophores	Case a11 1 hr. after 0.5 cc. adrenalin	Case a53 2 hrs. after 0.5 cc. adrenalin	Case a69 3 hrs. after 0.5 cc. adrenalin	Case a112 27 hrs. after 0.5 cc. adrenalin
Skin on back	Punctate	Punctate	Punctate	Stellate
Web	Punctate	Punctate to few stellate	Punctate	Stellate
Leg muscles	Complete expansion	Incomplete expansion. Few punctate.	Punctate	Punctate to incomplete expansion
Pleura	Complete expansion	Incomplete expansion	Stellate	Stellate to incomplete expansion
Pericardium	Complete expansion	Complete expansion	Incomplete to complete expansion	Stellate to complete expansion with proximal concentration
Muscles of body wall	Complete expansion	Incomplete expansion. Some punctate.	Punctate	Punctate
Mesenteries	Complete expansion	Incomplete expansion to few punctate	Punctate	Stellate
Back muscles	Complete expansion	50% punctate, 50% incomplete to complete expansion	Punctate. Occasional stellate.	Chiefly punctate. Some incomplete expansion.

In three individuals placed in a white dish for a day, there was a marked trend toward the contracted state among the internal melanophores. (Table I, third column.) As in the frogs kept on a dark background, there were all stages in the proximal withdrawal of the pigment. Likewise, animals kept on a white background for a week or

more showed great variety in the amount of contraction. (Table I, fourth column.) However, a greater general contraction usually existed with the longer period in white surroundings, suggesting a more sluggish response in the internal melanophores. Though not extreme, the contrast in the position of the pigment of these cells was evident in seventeen frogs kept in white dishes for at least two days, and in a like number confined in black jars for a similar period. (Diagram 1, top row.)

TABLE III

The response of melanophores to pituitrin. See also Table II, fifth column, for condition such as probably existed before the pituitrin injection in a113. Also, see Table I, second and third columns, for the condition of melanophores such as probably existed before the injections of pituitrin in a99 and a105 respectively.

Location of melanophores	Case a113—Pituitrin following adrenalin	Case a99—Pituitrin after 24 hrs. in dark	Case a105—Pituitrin after 2 days on white illuminated background
Skin on back	Complete expansion	Complete expansion	Complete expansion
Web	Complete expansion	Complete expansion	Complete expansion
Muscles of leg	Incomplete expansion	Incomplete expansion	Incomplete expansion
Muscles of back	Incomplete expansion	Incomplete expansion	Incomplete expansion
Pericardium	Incomplete to complete expansion	Complete expansion	Incomplete expansion
Pleura	Incomplete expansion	Incomplete expansion	Stellate to incomplete expansion
Mesenteries	Punctate to incomplete expansion	Incomplete to complete expansion	Incomplete expansion
Muscles of body wall	Incomplete expansion	Incomplete to complete expansion	Incomplete expansion

Adrenalin, which acts promptly and invariably on the dermal melanophores, also effected a slower contraction of the internal pigment cells. An injection of 0.5 cc. of a 1 : 1,000 solution of adrenalin chloride (Parke, Davis and Company) in the dorsal lymph spaces of eight frogs kept on a dark background for some days to insure expansion of these cells, evoked no reaction in the deep-seated melanophores for more than an hour (Table II, second column). Yet, the same dosage always caused the skin to become extremely light-colored within twenty

minutes. While a considerable percentage of the melanophores in the fascia of the back muscles of seven frogs contracted within an hour after an injection of 0.8 to 1.0 cc. of adrenalin, the effect of increasing the dose on the deep-seated melanophores was not pronounced. If, however, two hours elapsed between a 0.5 cc. injection and the inspection of the internal tissues (Table II, third column), as noted in four cases, at times as many as half the melanophores were entirely contracted, with a greater number among the pigment cells in the fascia of the back muscles. On increasing the period between the injection and the subsequent examination to three hours (Table II, fourth column), the contracted phase became general save in the pericardium. Here, the pigment migrated proximally only after many hours, as was noted in three or four frogs kept alive for nearly a day after the injection (Table II, fifth column). The dose of adrenalin was controlled in every case by an equal one of tap water. Frogs so treated never exhibited as great a contraction of the melanophores as those with adrenalin (Diagram 1, middle row). In fact, the stage of expansion was essentially similar to that in frogs kept in black jars without injections.

As an expansion of the melanophores is usual in the laboratory, and as their contraction is brought about so slowly, it was a bit uncertain in the pituitrin tests, whether a contraction of these cells had occurred in the individual frogs before injecting the drug effecting expansion. Since adrenalin was certain to produce a general proximal migration of pigment, it at first seemed desirable to inject it the day before the pituitrin. This procedure produced a complete expansion of the internal melanophores in eleven frogs, three hours after the injection of 0.5 cc. pituitrin (Parke, Davis and Company—"Obstetrical"). As an interaction of the drugs might have caused this, another test became preferable. Darkness was quicker and more certain in obtaining a contraction of the internal melanophores than white illuminated surroundings (Table I, second and third columns). In frogs confined in the dark for several days, a 0.5 cc. injection of pituitrin effected a more or less complete expansion in about three hours (21 cases) (Table III). In controls with the same amount of tap water, the melanophores were predominantly in the punctate or stellate condition (Diagram 1, bottom row).

Tadpoles

In the tadpoles of *Rana clamitans* in their second year, stubby relatively unbranched melanophores are numerous in most connective tissues, particularly in the peritoneum, the mesenteries, the pleura, and the pericardium (Pl. III).

When the tadpoles were confined in black jars for a day or more, the sub-epidermal melanophores almost always (22 out of 25 cases) exhibited a uniform state of maximum expansion. This condition was extended to the deep-seated melanophores in the pleura, the pericardium, the mesenteries, and, to a certain extent, to those of the peritoneum. In the latter tissue, however, in about half the tadpoles, some of these cells were in the stellate condition, while in one case the greater proportion of these was punctate.

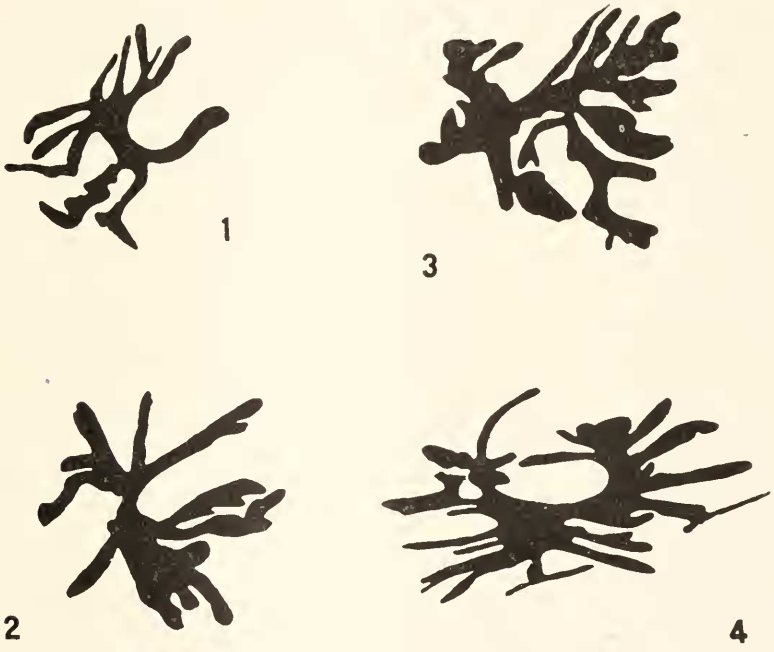

PLATE III

Semidiagrammatic drawings of completely expanded melanophores of tadpoles in (1) the pleura, (2) the mesenteries, (3) the peritoneum, and (4) the pericardium.

If tadpoles were kept in white dishes for a similar period, the sub-epidermal melanophores were usually (23 out of 25 cases) entirely contracted. Among the internal melanophores the amount of contraction which took place under these circumstances was not so great. Except for the melanophores of the pleura, which were predominantly contracted, there were many which exhibited a more or less distal distribution of pigment (Diagram 2, top row).

In larvæ, whose deep-seated melanophores responded as promptly as those in the skin to changes in background, adrenalin was a slower agent for contracting such cells than in the adult frog. Three hours after a

0.3 cc. injection in a 1 : 1,000 solution into the body cavity, the subepidermal melanophores ranged from the punctate stage to that of incomplete expansion and the internal cells remained for the most part completely expanded. Not until five hours (26 cases) after such a dosage were the majority of the skin melanophores contracted and even then a large percentage were in the stellate condition. Essentially the same situation was found in the internal melanophores. The proportion of animals with these cells in a punctate or stellate condition was greater than in tadpoles on a white background. With longer periods between the injection and the inspection of the animal, no great change occurred in the amount of contraction, and with a larger dose, the animal died within twenty-four hours. In controls with tap water, all the melanophores save a few in the peritoneum of about one-third the animals were completely expanded (Diagram 2, middle row).

With 0.3 cc. of pituitrin injected five hours before examination, the melanophores of tadpoles kept in white dishes were almost always (20 out of 25 cases) in the completely expanded phase. In the controls with tap water, there were only a few (5 out of 25 cases) with completely expanded melanophores (Diagram 2, bottom row).

Fundulus

When compared with the number of deep-seated melanophores in

Diagram 2. Tabulation of the effects of background, adrenalin, and pituitrin on the melanophores in five different parts of the tadpole: the dermis, the pleura, the pericardium, the mesenteries, and the fascia of the body wall muscles (as shown at the top of the diagram).

In the background tests, the shade of the surroundings, white or black, is indicated by *W* or *B* at the head of each column in the upper row. In these tests, as in all subsequent ones, the results of the factor effecting the more complete contraction are placed to the left and that producing the less complete to the right in each contrasting pair. Twenty-five tadpoles were kept on each background for forty-eight hours before observation. In every tadpole, the amount of melanophore contraction was noted as belonging to one of four stages of contraction of which 1 is maximum contraction and 4 minimum contraction. The number of cases falling in each class is recorded in black columns over the appropriate figure. Thus, in the dermis of tadpoles on a white background, 23 showed complete contraction, 1 part contraction, and 1 part expansion; on a black background, 22 exhibited complete expansion, 2 part expansion and 1 part contraction. The remaining tests are all tabulated in a similar fashion. In the adrenalin tests, both the tadpoles treated with a 0.3 cc. injection of the drug five hours before examining and their controls with 0.3 cc. tap water injections are designated *A* or *C* respectively at the top of each column in the second row. The 26 tadpoles in each series were kept on an illuminated black background for two days before observation. In the pituitrin tests, the tadpoles subjected to a 0.3 cc. pituitrin injection five hours before examination and their controls with 0.3 cc. of tap water are distinguished in the diagram by *P* or *C* at the top of each column in the bottom row. The 26 tadpoles in each series were confined in white dishes for several days before their final inspection

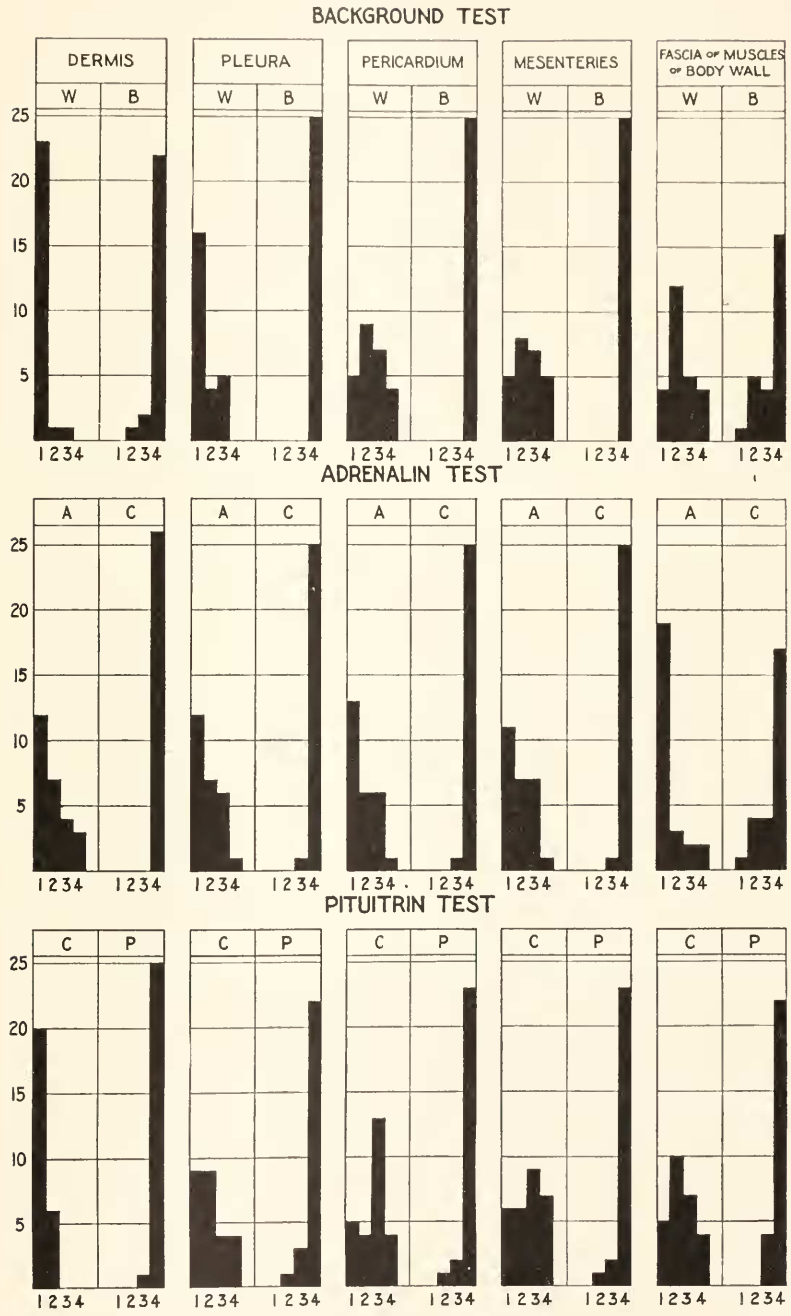
BACKGROUND TEST
DERMIS
PLEURA
PERICARDIUM
MESENTERIES
FASCIA OF MUSCLES OF BODY WALL
W
B
25
20
15
10
5
1234
ADRENALIN TEST
A
C
PITUITRIN TEST
C
P

Diagram 2

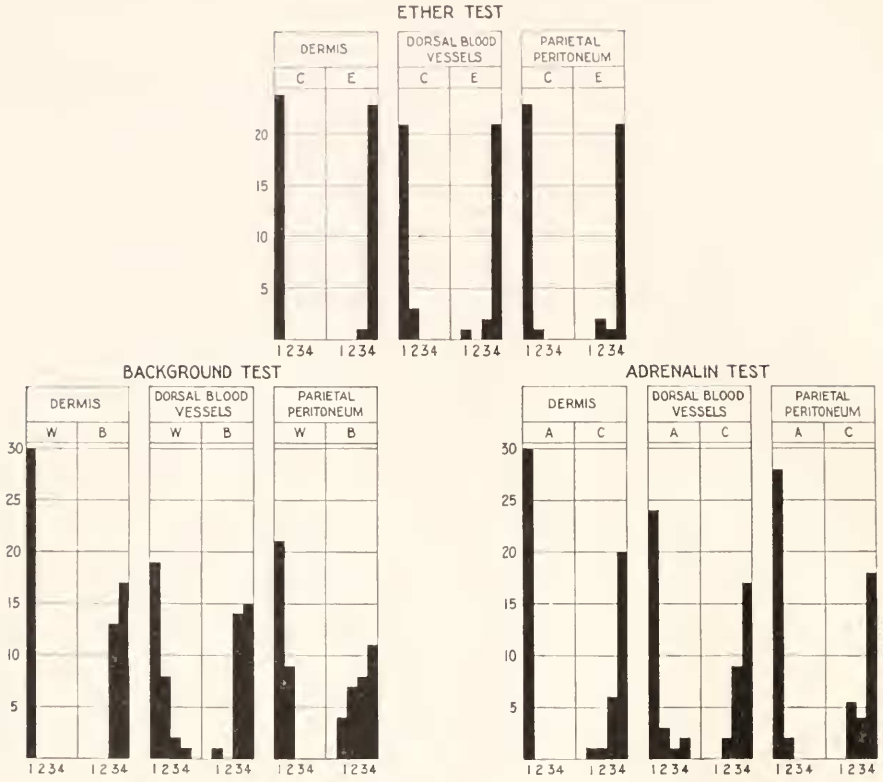

Diagram 3. Tabulation of the effects of ether, background, and adrenalin on the melanophores in three different parts of the killifish: the dermis, the connective tissue covering of the dorsal blood vessels, and the parietal peritoneum (as shown at the top of each column).

In the ether tests, the fish with a few cubic centimeters of this drug added to the water fifteen minutes before their examination and their controls in water alone are distinguished in the diagram by *E* or *C* at the top of each column in the upper row.

In these tests, as in all subsequent ones, the results of the factor effecting the more complete contraction is placed at the left and that producing the less complete to the right in contrasting each pair.

Forty-eight fish were kept in white dishes for a day before observation, of which but half were treated with ether. In every fish, the amount of melanophore contraction was noted as belonging to one of four stages of contraction of which 1 is maximum contraction and 4 minimum contraction. The number of cases falling in each class is recorded in black columns over the appropriate figure. Thus, in the dermis of fish kept on a white background, 24 showed complete contraction: on a white background with ether added to the water, 1 showed part expansion, and 23 complete expansion. All the remaining tests are tabulated in a similar fashion.

In the background tests, the shade of the surroundings, white or black, is indicated by *W* or *B* at the top of each column in the lower left-hand chart. Thirty fish were kept on each background for a day before observation.

In the adrenalin tests, both the fish treated with 0.2 cc. injection of the drug half an hour before examining and their controls with 0.2 cc. tap water injections are designated *A* or *C* respectively at the top of each column in the lower right chart. The 30 fish in each series were kept on an illuminated black background for a day before observation.

amphibia, those in *Fundulus* are scarce. Those in the mid-dorsal part of the parietal peritoneum and in the connective tissue of the dorsal blood vessels are large sheet-like cells. Those in the latero-dorsal portions of the parietal peritoneum and the more dorsal parts of the mesenteries and visceral peritoneum branch radially. In the visceral peritoneum, the true melanophores were usually obscured by melanin-filled syncytial cells—the " Pigmentflecken " of Ballowitz (1920). (Pl. IV.)

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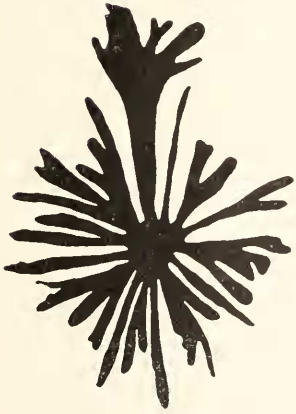

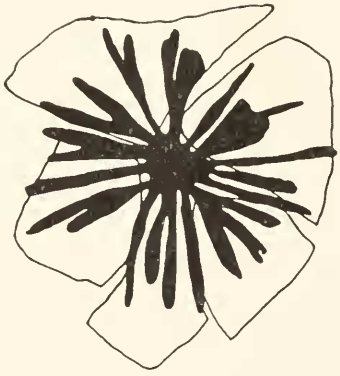

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PLATE IV

Semidiagrammatic drawings of completely expanded melanophores of the killifish in (1) the connective tissue covering the dorsal blood vessels, (2) the parietal peritoneum, and (3) the visceral peritoneum (including the syncytial melanin-filled cells in outline).

All the melanophores started to contract when the fish were transferred to white surroundings and a day in them insured complete contraction in the majority of the cells (30 cases). Indeed, expansion of these indicated a slow dissection. Although not always completed for several days, there was an immediate distal migration of the pigment in all the melanophores with a change to the black jars (30 cases) (Diagram 3, lower left-hand part).

Even more rapid changes may be effected by injecting 0.2 cc. of adrenalin into the body cavity or tail muscles. Within half an hour the melanophores were well contracted (30 cases) and this condition persisted even after killing the fish, except in some of the cells in the connective tissue of the dorsal blood vessels (Diagram 3, lower right-hand part).

Pituitrin does not affect killifish melanophores. Hence, ether, which produces an expansion of the dermal melanophores (Wyman, 1924), was used instead. The addition of a few cubic centimeters to the water in which the fish were swimming over a white background, proved to exert the same effect on the internal melanophores (Diagram 3, upper part).

Discussion

The similarity in appearance of the deep-seated and dermal melanophores suggests the occurrence of the same activities in both sets of cells. Nevertheless, earlier work seemed to show that this was seldom the case. Yamamoto (1931), in working on the peritoneal melanophores of a Japanese minnow, obtained little, if any support for a nervous control of such cells. However, since the melanophores which he described and figured were quite different from those in the dermis, they were probably the " Pigmentflecken " studied by Ballowitz (1920) rather than true melanophores, since these are scarce, if not entirely lacking in the peritoneum of many species. These may also be the cells Gilson (1926) observed in adult *Fundulus*. In the newly-hatched larvæ, he noted responses in the true melanophores apparently before they were masked by the development of these syncytial cells, and came to the conclusion that they were under nervous control. Later, in full grown killifish, as the expanded state usually persisted in the internal melanophores despite changes in the external coloring of the fish, he considered that there was little evidence for such control. Thus it appears likely that he actually recorded the inactivity of the " Pigment-flecken." In adult *Fundulus* there are few melanophores in the more dorsal portions of the visceral peritoneum, and these are not easy to find except in fish deficient in pigmentation. For this reason, most of the observations in this report were confined to the melanophores of the parietal peritoneum. In this region, the cells were found to respond synchronously with those of the dermis. These findings, with Gilson's results on the larvæ, make it appear likely that the deep-seated melanophores of the killifish, like those in the skin, are chiefly under nervous control.

In amphibians the earlier work also furnished little evidence for any activity in the deep-seated melanophores, but for quite different

reasons. Lieben (1906) and Uyeno (1922) tried the effects of adrenalin by perfusing the tissues with it or by its injection a short time before killing the frog. In some cases they recorded a slight proximal migration of the melanin. It now appears that the cause of this apparent inactivity is due to the unusually slow response of these cells. In my experience, the deep-seated melanophores of frogs did not react for at least three hours and those of tadpoles for nearly five hours. The observations of Lieben and Uyeno were made on animals which had been killed before the application of the drug or soon after its injection. Hence there was time for disintegration of the melanophores to set in before they could have responded to the drug. Indeed, it was natural to suppose that, if the drug calls forth any response in the deep-seated melanophores, it would do so in about the same time as it does in the dermis. Yet this never happens. Does this slower response mean that the dermal and internal cells are not controlled by the same agencies? In view of the fact that all the melanophores of fishes are excited by the same means, it would be peculiar to find that the deep-seated and dermal melanophores of amphibians are activated along different pathways, especially as they all react more or less slowly. The dermal melanophores of animals belonging to this group, unlike those in fishes, are known to be predominantly influenced by humoral agencies (Hogben, 1924). If we assume a similar situation to exist among the deep-seated melanophores, what is the explanation of the delayed response in these cells? Since the activating materials are carried in the circulation, there might be differences in the blood supply, on the one hand, to the dermal and, on the other, to the deep-seated melanophores. Because the skin is an accessory respiratory organ, there is probably plenty of oxygen available there. On the contrary, within the animal, there may be less of it and more carbon dioxide. Some observations have been made on the diverse effects of these two gases (Lowe, 1917; Uyeno, 1922; Wyman, 1924). However, their action is seemingly not a factor, because in the end the responses of the two sets of melanophores are essentially similar. A more likely explanation may be found in the distribution of the blood vessels themselves in the respective parts. While the skin is ramified by a network of veins and arteries, the internal connective tissues are supplied by relatively few vessels. Thus, unlike the skin, the melanophores in the deep-seated portions of the animal are often more remote from the blood supply. Not only would it take longer for the humoral materials carried in the blood stream to reach most of the melanophores on account of the longer portage in the lymph system, but these would also become progressively diluted by the lymph the farther away their source in the blood. The former

situation may perhaps be the cause of the slower rate of response of the deep-seated melanophores. The latter, since the cells are not equidistant from the blood vessels, would account for the less extreme and more variable states of expansion found among them. In this way, the differences in the response of the dermal and internal melanophores may be explained. Accordingly, it is probable that the deep-seated melanophores are also controlled by humoral agencies.

The occurrence of similar activities in the dermal and deep-seated melanophores brings up the question of the function of these cells. Many representatives of the lower vertebrates by an expansion or contraction of the dermal melanophores, mimic, to some extent, the shade of their surroundings. This is often interpreted as being a protective measure or an aid in capturing their prey. If this be so, it seems odd, at first, that such changes should also take place within the animal. However, when we consider how sluggish the response is in the deep-seated melanophores of frogs and tadpoles, it is unlikely that the changes in the dermal cells are often reflected in the deeper portions of the animal. Accordingly, it is hard to believe that these cells by reason of their activities are ever of any particular use to the animal. In *Fundulus*, on the other hand, where the internal melanophores may be expected to react synchronously with every fleeting color change in the skin, there are so few true melanophores that it is difficult to conceive of their ever playing a very great rôle. Thus, either on account of their sluggish responses or their scarcity, the activities of the deep-seated melanophores are insignificant as compared with those in the skin. Hence, the fact that the deep-seated melanophores may display many of the activities of those in the skin does not make it necessary to dispense with the usual idea that the function of color change is an adaptive one.

Summary

In frogs, the deep-seated melanophores of the fascia of the leg muscles, of the muscles of the back, of the muscles of the body wall, of the mesenteries, and of the pleura respond to white or black backgrounds, adrenalin, and pituitrin in the same manner as do those in the skin, but about six times more slowly.

In tadpoles, the deep-seated melanophores of the fascia of the body-wall muscles, of the mesenteries, of the pericardium, and of the pleura respond to white or black backgrounds, adrenalin, and pituitrin in the same manner as do those in the skin, but react about ten times more slowly to the drugs.

In killifish, the deep-seated melanophores of the parietal peritoneum

and the connective tissues of the dorsal blood vessels respond to white or black backgrounds, adrenalin, and ether synchronously with those in the skin.

It is suggested that the difference in the speeds of response of the deep-seated melanophores in fishes and in amphibians is dependent upon the two methods of control which exist in the dermal melanophores of these two classes—nervous for fishes and humoral for amphibians.

In amphibians, the difference in the speed of dermal and deep-seated melanophores is believed to be due to the distribution of the blood vessels. While the skin is ramified with small blood vessels, the deep connective tissue receives a relatively scanty supply, for while many large veins and arteries pass through this tissue, they give off few branches to these parts. Also, the less extreme and more variable condition found among the deep-seated melanophores is thought to be caused by a dilution of the activating materials by the lymph.

The similarity of response in the deep-seated and dermal melanophores does not reveal what function these cells may serve.

Although the internal melanophores obviously play no part in color changes, neither does their activity interfere with the usual idea that the function of color changes in the skin is an adaptive one, because they are either so few in number that their rôle must be slight (fishes), or so sluggish that they seldom reflect the changes of the skin (amphibians).

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